

Page No. _____

purpose: Amplification of pMC9 within different amounts of Tag + dilution. Deep Vent + Tag + Deep Vent all samples discarded

constant different conc dilution

using dU primers, 1 μM

200 μM dNTP

1 μM primer dU \times 2728 + 2729 (100 μM)

100 μg template diluted 100/ λ \rightarrow 1/ λ

Deep Vent buffer

Dilutions: a. Tag: 0, 0.5, 1, 1.5, 2, 2.5 and 5 λ

b. Tag: Deep Vent: 1.0, 1:0.001, 1:0.005, 1:0.01, 1:0.05
1:0.1, 1:0.2, 1:0.5, 1:1, 1:2

c. Deep Vent: 0, 0.025, 0.05, 0.1, 0.5, 1, 2, 5 λ

prepared a master mix w/o enzyme. Calf added separately.

Did just one of each.

11/20 1167

10 x buffer 150

primer 15

2 15

Tag: 5 λ / λ diluted to 10/ λ in 1x buffer. Template 3 μl

Template H₂O

0	=	-	5	D.V	20/ λ	\rightarrow	10/ λ	\rightarrow	0.10/ λ	45 μl
1.5	=	0.5	4.5							0.0010/ λ
1	=	1	4	0.025	2.5					0.0010/ λ
1.5	=	1.5	3.5	0.05	5					0.0010/ λ
2	=	2	3	0.1	1					0.0010/ λ
2.5	=	2.5	2.5	0.5	5					0.0010/ λ
5	=	5.0	-	1.0	0.5	\downarrow	20/ λ	\downarrow		5.0 = 2.5 μl To Pag No.
				2.0	=	A				

8 - 15

Ised & Understood by me,

Date

Invented by

Date

11/18/94

Recorded by

11/18/94

K. Sitaraman

Project No. _____

100

Book No. _____

TITLE _____

From Page No. _____

T	D.V.	Taq	W/x	D.v	dnTTP	in	Tube #x
Unit	1	0	1	μl	1	1	16
		0.001			5	1.001 μl/x	17
		0.005			1	0.1 μl/x	18
		0.01			5	0.1 μl/x	19
		0.05			1	0.1 μl/x	20
		0.1			5	0.1 μl/x	21
		0.5			1	0.1 μl/x	22
		1.0			5	0.1 μl/x	23
		2.0			1	2.0 μl/x	24

- Thermocycled at 94° , 5'

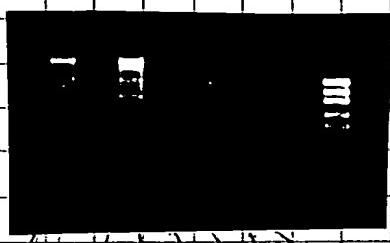
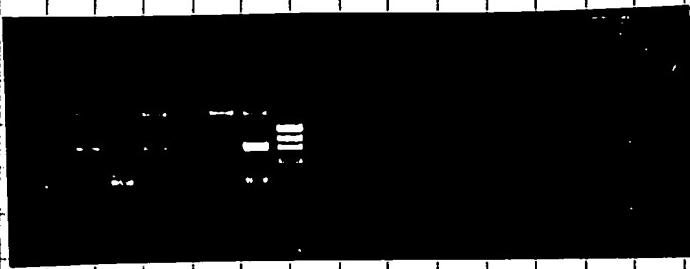
30 (94° , 30', 56', 45 sec) 72', 3')

- after 6 cycles noticed dNTP was not added (1) so added 1 μl of 10 mM dNTP (200 μM / 20 μl x) individually each tube + started again the cycling!

may be have to repeat again.

Taq alone Deep vent above

Taq : Deep vent



No contamination?
(no enzyme)

.001 .005 .05 .5 1

so much mispriming:

Repeat or in dry heat, use Lambda / 1+ in 1% marker

To Page 1

Witnessed & Understood by me,

Date
11/20/89

Entered by

Recorded by

R. Sreenanam

Date

11/21/89